induced a marked increase of MTT values compared to control culture. Time course experiments performed with chondrocytes cultivated in 30% platelet poor plasma (PPP) or in 30% PRP showed that PRP promoted an increase of MTT values that was observed for the entire 12 day testing period.
In PRP gel, cell bodies and pericellular matrix were stained more strongly with alcian blue compared with that in PPP gel. Likewise, immunoreactivity to the anti-type II collagen antibodies was shown more certainly in PRP gel. Histochemical and immunohistochemical examinations demonstrated that cultivated chondrocytes can easily lose their phenotype even in passage 1 cells, however PRP enhances redifferentiation of dedifferentiated chondrocytes.
Conclusion: 30% PRP promotes human chondrocyte proliferation. Cells expanded with 30% PRP can express chondrocyte phenotype. It is suggested that PRP gel provides an invaluable source of growth factors for human chondrocyte proliferation and redifferentiation, and could serve as a scaffold for autologous chondrocyte implantation that has potential availability for repair of osteoarthritis with chondral defects.
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A NOVEL INTERACTION BETWEEN COMP AND GRANULIN-EPITHRIN PRECURSOR (GEP): POTERNTIAL EFFECTS ON MUSCULOSKELETAL TUMORS GROWTH

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Study aims: Granulin-epithelin precursor (GEP) was identified as a COMP-binding protein in a functional genetic screen. GEP is a secreted growth facotor. It contributes to tumorigenesis in diverse cancers. The aim of this study was to characterize the interaction between GEP and COMP and to investigate their potential as therapeutic targets in musculoskeletal tumors.
Methods: *Yeast two hybrid (Y2H) Library Screen.* Functional domains of COMP were used as bait to screen rat brain pPC86 cDNA library. *In VitroBinding Assay* – Glutathione-Sepharose beads preincubated with either GST or GST-EGF domain of COMP were incubated with purified His-tagged GEP. Bound proteins were detected with anti-His antibodies. *Proliferation Assay* – rat chondrosarcoma cells (RCS) were transfected with empty vector, construct encoding GEP and constructs encoding GEP and COMP respectively and selected with G418. Cell proliferation was assayed by seeding $1x10^5$ cells into 6-well plates. Cells were harvested and viable cells were counted.
Results: *Isolation of GEP as a COMP binding partner.* To better understand the biological functions of COMP, we performed Y2H screen. Briefly, a Y2H rat cDNA library was screened with the construct encoding the EGF-like repeats of COMP. We screened approximately 2.5 million clones and identified 12 positive clones. Two positive clones among them encoded two N-terminal truncated mutants (a.a. 228-588; a.a.334-588) of GEP (Accession #NM_017113.1).
COMP directly binds to GEP – To verity the interaction between COMP and GEP which was first identified in yeast, a GST pull-down assay was performed to test whether EGF-like domain of COMP binds to the C-terminal of GEP (a.a.228-588) in vitro. GST did not pull down GEP protein, whereas GST-EGF efficiently pulled down GEP protein, indicating binding of COMP to GEP in vitro.
COMP enhances the GEP-mediated stimulation of chondrosarcoma cell proliferation – Overexpression of COMP alone produce negligible effects on tumor cell growth; whereas overexpression of GEP resulted in a clear increase in cell proliferation by 43% in day 3 and robust stimulation was observed in day 4 (1.8 fold) and day 5 (3.5 folds). Intriguingly this GEP-mediated stimulation was further enhanced by co-expression of COMP. The enhancement was observed since day 2 (2.3 folds) and remained significant with slightly going down (2.1 folds in day 3, 1.7 folds in day 4 and 1.5 folds in day 5 respectively).
Conclusion: We reported a novel interaction between a musculoskeletal tissues-specific extracellular matrix COMP protein and a GEP growth factor and the enhancement of GEP-stimulated chondrosarcoma growth by COMP. Since GEP was found to promote the growth, invasion and metastasis of several tumors, our findings demonstrate that COMP may function as an important mediator of musculoskeletal tumors via associating with GEP, presenting GEP to its membrane receptor and regulating GEP-mediated signal transduction and gene regulation pathways.
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CYCLOOXYGENASE-2 ACTIVITY IS REGULATED BY LIMITED PROTEOLYSIS

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Although OA is not considered an inflammatory arthropathy, it is now clear that inflammatory modulators are prominent players in pathogenesis of the disease. Overproduction of the immunomodulator prostaglandin E2 (PGE2) and of the rate-limiting enzyme involved in its biosynthesis, cyclooxygenase-2 (COX-2), by OA and RA chondrocytes and human synovial fibroblasts (HSFs) have made this enzyme the subject of intense interest. While an impressive number of regulatory mechanisms implicated in COX-2 gene expression are now known, the mechanisms regulating COX-2 catalytic activity are still not fully understood. We have uncovered a novel control mechanism involving limited proteolysis of the COX-2 protein that regulates intrinsic cyclooxygenase/peroxidase activities and ultimately the biosynthesis of PGE2. IL-1β stimulation of human synovial fibroblasts (HSFs), chondrocytes and macrophages induced the synthesis of COX-2 protein and its coincident proteolysis into a series of stable, distinct immunoreactive fragments (i.e., 66, 42-44, 34-36 and 28 kDa) that accumulated in a time-dependent manner. The induction of PGE2 synthesis (i.e., COX-2 activity) in HSFs coincided with the start of COX-2 fragmentation. Regression analysis confirmed a direct linear correlation (R2=0.91) between PGE2 secretion and the timing and degree of COX-2 proteolysis in IL-1β-treated HSFs, as well as cells treated with other arthropathologically relevant pro-inflammatory cytokines (i.e., TNF-alpha, IL-17). Cleavage positions within COX-2 were confined to the catalytic domain as confirmed by mass-spectrometry (MALDI-TOF). Pharmacological inhibition of induced COX-2 activity by a variety of non-selective and COX-2-specific NSAIDs abrogated proteolysis. NSAID-mediated inhibition of proteolysis was not mechanistically related to direct enzymatic inactivation of COX-2, as shown by the reduction in COX-2 cleavage and PGE2 synthesis by (R)-flurbiprofen, which does not inhibit COX-2 and is not efficiently isomerized to the active (S)-flurbiprofen. These results pointed to the involvement of NSAID-sensitive proteolytic enzymes in the COX-2 fragmentation process, a hypothesis supported by the correlated reduction in COX-2 cleavage and PGE2 synthesis by both g-secretase and cysteine/calpain protease inhibitors. Our results suggest that nascent COX-2 is initially in an enzymatically latent form and must be activated by limited proteolysis for full biosynthetic activity.